RNA preparation

We have found that the combined use of two commercially available RNA isolation kits produces consistently high quality RNA samples for microarrays. The protocol is a modification of the two protocols combining steps and saving time. This is not an endorsement of either product and we are confident that analogous kits from other vendors will produce high quality RNA.

1. Isolate RNA using Trizol Reagent.

- Suspend pelleted cell sample in 1ml of Trizol reagent for each 5-10 x10^6 cells. Pipette up and down. Some cell types are more difficult to lyse (e.g. yeast or mycobacterium) and may require mechanical disruption in the presence of Trizol reagent. Incubate 5 min.
- Add 0.2x volume (of Trizol used) of Chloroform. Shake tube well. Incubate 2 min.
- Centrifuge at 13,000 x g for 15 min at 4oC and collect upper clear layer.
- This is equivalent to taking the trizol protocol through the phase separation step and then transferring the aqueous phase into the Qiagen RNA clean-up procedure below.

2. Perform Qiagen RNA Clean up procedure

- Perform at room temperature. Add an equal volume of 70% Ethanol to the Trizol upper layer. Mix well and apply to RNeasy spin column. Check product information for capacity of columns.

- Centrifuge Discard flow through. Wash column 2x with RPE buffers supplied in the kit. Be certain ethanol has been added to RPE concentrate before use.

- After the last spin the column is dry so that the Ethanol is removed. Transfer the column to a fresh collection tube.

- Elute RNA with the designated volume of RNase free water. Allow to stand for 1 min then centrifuge collecting the eluted RNA. Repeat one time.

3. Determine RNA concentration

- RNA is eluted from Qiagen columns in water. Dilute a portion of the RNA 1:100 in TE [10mM Tris-HCl (pH7.5), 1mM EDTA (pH 8.0)] and read absorbance at 260nm and 280nm. An OD_{260} reading of 1 corresponds to 40μg/ml of RNA. The OD_{260}:OD_{280} ratio of the sample should be ~2.0.

4. Examine the RNA integrity.

- Run RNA sample on a 2.0% Agarose gel in TAE. The ribosomal RNA bands will not appear to be as crisp as those observed on a denaturing (formamide) gel but the TAE agarose gel will allow estimation of RNA integrity and purity.